Nucleic acid hybridization assays employing dA-tailed capture probes. Single capture methods.

Morrissey DV, Collins ML.

Gene-Trak Systems, Framingham, MA 01701.

Several novel hybridization techniques are described. Cells or specimens are treate to release nucleic acids and a liquid phase hybridization is carried out with a dA-tailed capture probe and a reporter probe in chaotropic salts or in salts containi SDS/proteinase K. In another format the tailed capture probe is preimmobilized on polystyrene and used to capture target nucleic acids from the solution. No phenol extraction or centrifugation is required to prepare the nucleic acids. Capture of the target on the poly (dT)-solid supports is used to remove excess labelled probe and sample impurities prior to non-radioisotopic or radioisotopic detection. This paper shows the advantage of a single round of capture on polystyrene, including the abil to assay large numbers of samples manually, the ability to analyse each sample for many analytes simultaneously, the use of rapid non-radioisotopic detection, and th ability to readily adapt the assay for automation.

PMID: 2671681 [PubMed - indexed for MEDLINE]

1: Anal Biochem 1989 Sep;181(2):345-359 Related Articles, Bool

Nucleic acid hybridization assays employing dA-tailed capture probes. I. Multiple capture methods.

Morrissey DV, Lombardo M, Eldredge JK, Kearney KR, Groody EP, Collins ML.

Gene-Trak Systems, Framingham, Massachusetts 01701.

A quantitative hybridization assay termed "reversible target capture" is described. The technique is designed to extensively purify the target nucleic acid from crude cell lysates in about 1 h without phenol extraction. Simple, rapid methods are described that explain how each process in the assay is optimized. The procedure involves hybridizing the target nucleic acid in solution with a dA-tailed capture probe and a labeled probe. The capture probe-target-labeled probe "ternary comple is then captured on magnetic beads containing oligo(dT). After the excess unhybridized labeled probe, cell debris, and other sample impurities are washed away, the intact ternary complex is further purified by chemical elution from the beads and recapture on fresh beads. The ternary complex is then eluted thermally a recaptured on a third set of beads or on poly(dT) filters. This triple capture method results in a detection limit of approximately 0.2 amol (100 fg) of target with 32P-labeled riboprobes. This is approximately 1000 times more sensitive than sandwich assays employing only a single capture step. The method is illustrated by detecting Listeria cells in the presence of heterologous bacteria. With three rounds target capture, as few as six Listeria cells have been detected in the presence of 1.2 x 10(7) control cells.

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1: Anal Biochem 1989 Sep;181(2):360-370 Related Articles, Bool

Nucleic acid hybridization assays employing dA-tailed capture probes. II. Advanced multiple capture methods.

Hunsaker WR, Badri H, Lombardo M, Collins ML.

Gene-Trak Systems, Framingham, Massachusetts 01701.

A fourth capture is added to the reversible target capture procedure of the precedin paper. This results in an improved radioisotopic detection limit of 7.3 x 10(-21) m of target. In addition, the standard triple capture method is converted into a nonradioactive format with a detection limit of under 1 amol of target. The princip advantage of nonradioactive detection is that the entire assay can be performed in about 1 h. Nucleic acids are released from cells in the presence of the ('capture probe') which contains a 3'-poly(dA) sequence and the ('labeled probe') which contains a detectable nonradioactive moiety such as biotin. After a brief hybridization in solution, the target is captured on oligo(dT) magnetic particles. Th target is further purified from sample impurities and excess labeled probe by recapture either once or twice more on fresh magnetic particles. The highly purifie target is then concentrated to 200 nl by recapture onto a poly(dT) nitrocellulose filt and rapidly detected with streptavidin-alkaline phosphatase using bromochloroindolyl phosphate and nitroblue tetrazolium. Using this procedure, as little as 0.25 amol of a target plasmid has been detected nonradioactively in crude samples in just 1 h without prior purification of the DNA and RNA. Finally, a new procedure called background capture is introduced to complement the background-reducing power of RTC.

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Erratum in:
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Immunodetection of DNA with biotinylated RNA probes: a study o reactivity of a monoclonal antibody to DNA-RNA hybrids.

Coutlee F, Bobo L, Mayur K, Yolken RH, Viscidi RP.

Eudowood Division of Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

A quantitative, nonisotopic hybridization assay which measures specific DNA-RN hybrids is described. A biotinylated RNA probe is reacted in solution with a DNA target and the labeled hybrids are immobilized onto a solid phase surface with an antibiotin antibody. Bound hybrids are detected with a beta-galactosidase-labeled monoclonal antibody against DNA-RNA hybrids and are quantitated with the addition of a fluorogenic substrate. In a model system using pSP65 or pGEM4 plasmids and transcripts, biotinylated RNA probes allowed detection of 5 pg of DN in 10(6) pg of exogenous nucleic acids in 1000 min. Signals generated in the syste depended on input target length. A nucleic acid target of 25 bases was still detectal in the assay. Human immunodeficiency virus type 1 (HIV-1) DNA was amplified i the polymerase chain reaction with Taq polymerase and a set of primers for the pol gene, one of which contained T7 RNA polymerase promoter sequences. A HIV-RN probe of 326 bases was transcribed with T7 RNA polymerase using polymerase chain reaction (PCR) amplified DNA as a template. The RNA probe of 326 bases performed as well as a RNA probe of 2588 bases for detection of a DNA segment 355 bp. For detection of dilutions of HIV-1 with PCR, a set of primers (outer set) was used for amplification of HIV-1 DNA. In a separate reaction a set of primers nested between the first set generated through PCR an amplified DNA fragment w the T7 promoter. This fragment was transcribed for the synthesis of a biotinylated RNA probe. This probe could then be reacted with material amplified with the out set of primers. Ten copies of HIV-DNA could be detected with this procedure.

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